

Dean's Scholars Honors Thesis

**Alcohol Consumption Promotes Breast Cancer Development in
Female Mice**

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ABSTRACT

Backgrounds: Epidemiological studies show that alcohol consumption increases breast cancer risk. However, the mechanism by which alcohol causes this effect is not known. Therefore, our objective is to generate an animal model to find the mechanism by which alcohol increases breast cancer risk.

Methods: To determine the effects of alcohol consumption on breast cancer development, 65 female FVB/N mice were randomized (30-water, 35-alcohol) to have free access to water or 20% alcohol. All mice were given the same diet and placed in similar living conditions. Eleven weeks into the study, mice were injected with Met-1 mammary cancer cells subcutaneously in their backs. We measured body weight, food, liquid consumption, and tumor growth rate throughout the study. To determine body composition, mice were scanned using a GE Lunar Piximus Densitometer, which measures percent body fat, percent lean body mass, and bone mineral density.

Results: Results showed that tumor development is exacerbated in the alcohol consuming mice. Alcohol consuming mice developed tumors earlier than water consuming mice. Furthermore, tumors in the alcohol consuming mice were larger in volume. Body weights, caloric consumption, and body composition showed no significant difference between the alcohol and water consuming mice.

Conclusion: We present a suitable animal model where we can elucidate the mechanism by which alcohol consumption promotes mammary tumor development.

BACKGROUND

Breast cancer is the most common cancer in women in the U.S. and the second leading cause of death. (1) An estimated 40,460 women are expected to die from the disease in one year alone and over 150,000 new cases are diagnosed each year. (2)

There are several non-modifiable factors that increase breast cancer risk, including age, family history, age at first full-term pregnancy, early menarche, and delayed menopause. (2, 3) However, other risk factors can be controlled by behavioral and dietary changes. (4) These modifiable risk factors include alcohol consumption (increased risk), body weight (obesity; increased risk), and exercise (decreased risk). (2) Our present objective is to determine how alcohol affects breast cancer development.

Alcohol consumption increases breast cancer risk in a dose-dependent manner. (5, 6, 7) For example, observational studies show that one or two drinks per day increases breast cancer development in women by 30-50%. (8) Furthermore, evidence shows that regions with greater alcohol intake (more than 1-2 drinks per day) have higher breast cancer incidence. (1) Epidemiological studies suggest that breast cancer risk increases by 7.1% with each additional 10g/day intake of alcohol. Data showed that women who drink 35-44 g/day of alcohol had a 32% higher chance of developing breast cancer than those who don't drink. Women consuming up to 45g/day of alcohol showed almost a 50% higher risk for breast cancer. (9) Thus the evidence shows that alcohol consumption concretely increases breast cancer risk.

The mechanism by which alcohol consumption exacerbates mammary tumor development is not known. However, several hypotheses have been suggested to explain the means by which alcohol affects breast cancer risk. Studies suggest that alcohol increases breast cancer risk by increasing the production of reactive oxygen species (ROS) which can contribute

to DNA damage (1). Another suggested hypothesis indicates that alcohol may increase breast cancer risk by increasing the levels of the hormone estrogen. (10, 11) However, review of the literature suggests that alcohol is not a direct carcinogen to people, but rather behaves as a co-carcinogen or tumor promoter. (12)

Epidemiological studies clearly show that alcohol consumption increases breast cancer risk. (13, 14) However, human studies have not been able to determine the mechanism by which alcohol increases breast cancer risk. Animal studies can help us elucidate the mechanism by which alcohol increases breast cancer risk. In animal studies, factors that can confound the alcohol-breast cancer relationship can be controlled. For example, in some epidemiological studies, recall bias may confound the alcohol-breast cancer relationship. When measuring alcohol consumption in human studies, the investigator relies on the memory of the individuals to accurately remember how much they drink. This may not be reliable because of recall bias. (15) However, in animal studies, we can specifically control for alcohol consumption, diet, age, genetics, and other possible confounders that may affect the alcohol-breast cancer relationship.

Therefore, the purpose of this study is to use female mice as a model to determine the effects of alcohol consumption on breast cancer development. Having a mouse model of breast cancer will allow us to specifically determine if indeed alcohol consumption promotes tumor development through factors such hormones or reactive oxygen species (ROS) and control for confounding factors such as age and genetics. Determining how alcohol promotes breast cancer development may provide us with targets in order to prevent or treat alcohol related breast cancers.

METHODS AND PROCEDURES

Animals

Sixty-five six-week old FVB/N female mice were purchased from Jackson Laboratory (Bar Harbor, Maine). Mice were allowed to adjust to their new surroundings for one week. Then, the mice were randomized to a water group or an alcohol group. All mice had free-access to a 5% low fat diet and to either water or 20% alcohol (mice consumed approximately 20-30 grams of alcohol per day or about 3 beers daily).

Alcohol Consumption

Previous studies in our laboratory showed that young mice do not like 20% alcohol. Therefore, we used an alcohol gradient treatment approach to adjust the mice slowly to alcohol consumption. Initially, mice were given 5% alcohol for one week, then 10% for the following three weeks, 15% for another three weeks, and finally 20% alcohol. For the rest of the study, all mice were given either free-access to 20% alcohol or water.

Food and Liquid Consumption

The 5% low fat diet consisted of 19.2% protein, 67.3% carbohydrate and 4.3% fat (Research Diets Inc., D12350B). Food intake was monitored weekly using a Mettler Toledo PL601-S balance. Liquid was administered in 50mL centrifuge tubes (Corning) with appropriate bottle caps for mice. Again, all mice had free access to either water or 20% alcohol. Liquid was measured once a week using a Mettler Balance.

Body Weight

Body weights were measured weekly using a Mettler Toledo balance. We also used body weight as an indication that all mice were healthy.

Cancer Cells

Mice were injected with Met-1 mouse mammary cancer cells. These breast cancer cells were originally obtained from mammary tumors dissected from polyomavirus middle T antigen transgenic mice (PyMT). PyMT mice express the polyomavirus middle T antigen transgene in the mammary gland under the control of a mouse mammary tumor virus promoter/enhancer. (16) Expression of the transgene predisposes mice to development of mammary tumors (16, 17). Female mice develop mammary carcinomas by 8-10 weeks. The cancers in these mice metastasize to the lungs at a frequency of >80%. The genetic alterations in these tumors are similar to those found in human breast cancers, such as HER-2 (c-erbB2) over-expression. (18) PyMT transgenic mice are genetic descendants of FVB/N mice so tumor cells isolated from PyMT mice can be transplanted into our FVB/N mice through injection. Thus, to determine the effects of alcohol consumption on mammary tumor development, 50,000 Met-1 breast cancer cells were injected subcutaneously in the backs of the mice consuming water or alcohol. The alcohol group FVB/N mice had been consuming alcohol for approximately 11 weeks when the tumor cells were injected.

Monitoring Tumor Growth

To detect the appearance of tumors, mice were palpated three times a week. Once tumors became palpable, tumor volume was determined by measuring the length, width, and depth of the tumors three times a week using calipers (Fischer-Scientific Digital Calipers).

Body Composition

After tumors were dissected out of the mice, the remaining mice bodies were put in plastic body bags and preserved in a -20 degree fridge. The mice bodies were eventually scanned using a GE

Lunar Piximus Densitometer to determine any differences between the water and alcohol consuming mice in terms of bone density and tissue composition.

RESULTS

Tumor Size and Volume

Results from the study show that alcohol increases mammary tumor development. Figure 1 shows that tumors were detected earlier in mice consuming alcohol. At the end of the study, 97% of the alcohol mice had palpable tumors compared to only 60% in water consuming mice. Furthermore, Figure 2 show that for alcohol consuming mice developed bigger tumors than the water consuming mice. At the end of the study, average tumor volume for alcohol consuming mice was approximately 2300 mm³ and about 700 mm³ in the water consuming mice. These results show that alcohol significantly exacerbates mammary tumor development in female mice.

Food and Liquid Consumption

Food consumption (Figure 3) between alcohol and water consuming mice was similar at the beginning of the study. However, in the latter half of the study, alcohol consuming mice consumed less food than water groups. Alcohol mice consumed an average of 0.4 grams less food than water consuming mice. However, total calorie intake was not significantly different between water (food calories) and alcohol (food + calories from alcohol) consuming mice (Figure 4).

Body Weights and Composition

Figure 5 shows that body weights were similar between the two groups throughout the study. Body composition was determined using a GE Lunar Piximus Densitometer. These analyses determined percent body fat, lean mass content, bone mineral density and bone mineral content (Figure 6a and 6b). There was no significant difference in %body fat and lean mass between the

two groups (Figure 6a). For example, water consuming mice had 23.4% body fat and 76.7% lean mass and alcohol consuming mice 23.8% body fat and 76.2% lean body mass. However, bone mineral density was lower in the alcohol consuming mice ($p < 0.05$) (Figure 6b).

DISCUSSION

Results show that alcohol consumption increases mammary tumor growth in female mice. The data shows that alcohol consuming mice developed palpable tumors earlier and had larger tumor than water consuming mice. At the end of the study, 60% of the water consuming mice had palpable tumors, while 97% of the alcohol mice had palpable tumors. Moreover, tumors were bigger in the alcohol consuming mice; the tumors in the alcohol consuming mice were approximately 1287mm^3 larger than the tumors in the water consuming mice.

Total calorie intake was not different between the water and alcohol mice. Alcohol consuming mice had a slight decrease in food intake; however, total calorie intake was not different between the two groups. Therefore, the data shows that the alcohol mice replace part of their caloric intake with calories from alcohol. Body weights were not different between the two groups. Body composition analyses show that body fat and lean mass was not different between alcohol and water consuming mice, therefore, it is unlikely that alcohol affects breast cancer development by affecting body composition.

In summary, we show that alcohol consumption promotes tumor development in female mice. This mouse model of breast cancer may allow us to determine the mechanism by which alcohol promotes breast cancer development.

FUTURE EXPERIMENTS

While our experiments show that alcohol does increase breast cancer development in mice, further experiments need to be done to provide more explanation towards the details of this

phenomenon. For instance, using this model for breast cancer development, we may be able to determine if alcohol promotes tumor development by exacerbating inflammation or by promoting angiogenesis (blood vessel formation). If the alcohol mice have higher levels of angiogenesis in the tumors this may explain why the tumors grow at a faster rate in the alcohol consuming mice. Likewise, it may be possible that alcohol may promote tumor development by exacerbating inflammation, which has been associated with breast cancer.

ACKNOWLEDGEMENTS

I would like to thank Dr. Nomeli Nunez for being a wonderful supervisor and advisor towards my research experience at the University of Texas. His support and patience really helped me realize what it means to do independent research. I would furthermore like to thank Jina Hong for graciously allowing me to take part in her experiment and for allowing me to write an undergraduate thesis on the topic. (It should be noted that the data presented in this thesis will eventually be submitted by Jina Hong and published in *Alcoholism: Clinical and Experimental Research*). Also, I am very grateful for all the help that Jina Hong extended to me throughout the research and the countless number of occasions where she took time away from her busy schedule to give me advice and suggestions. In addition, I want to thank the Dean's Scholars program and the extraordinary people who work so hard to keep it running so smoothly.

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Figure 1: Percentage of mice with palpable tumors after tumor injection

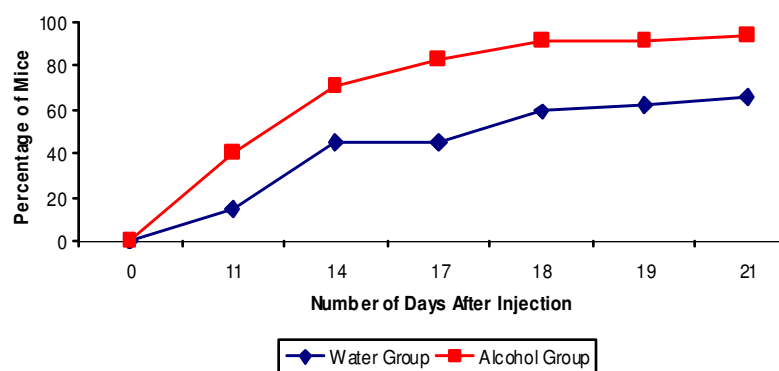


Figure 2: Tumor Volume

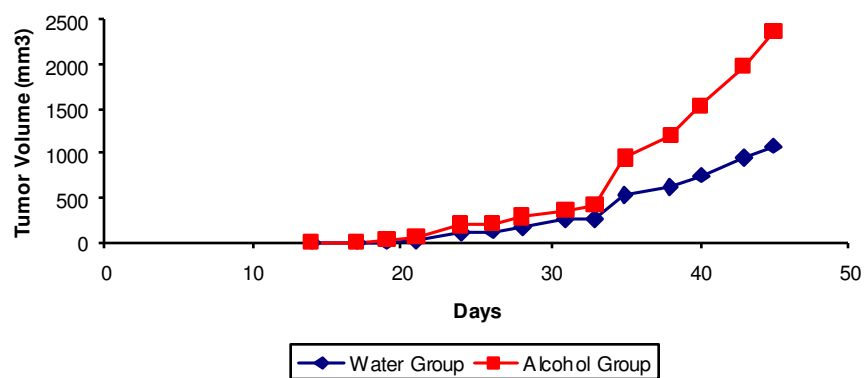


Figure 3: Food Consumption

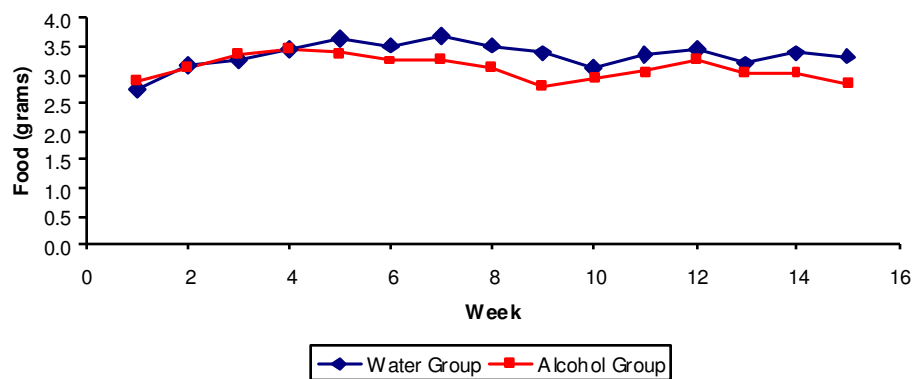


Figure 4: Total Calorie Consumption

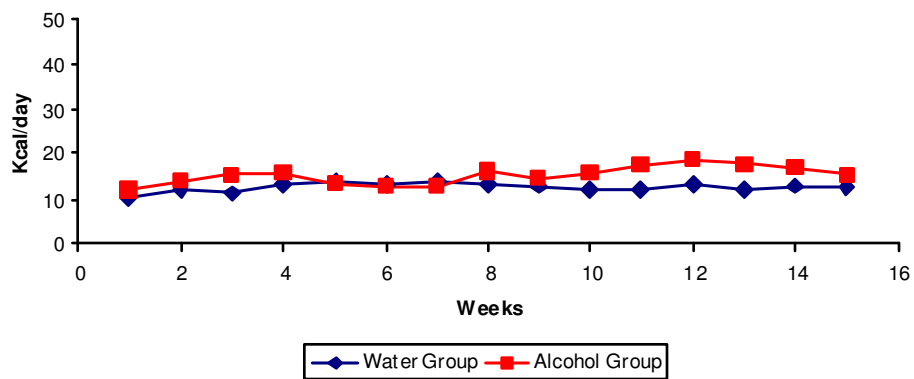


Figure 5: Body Weight

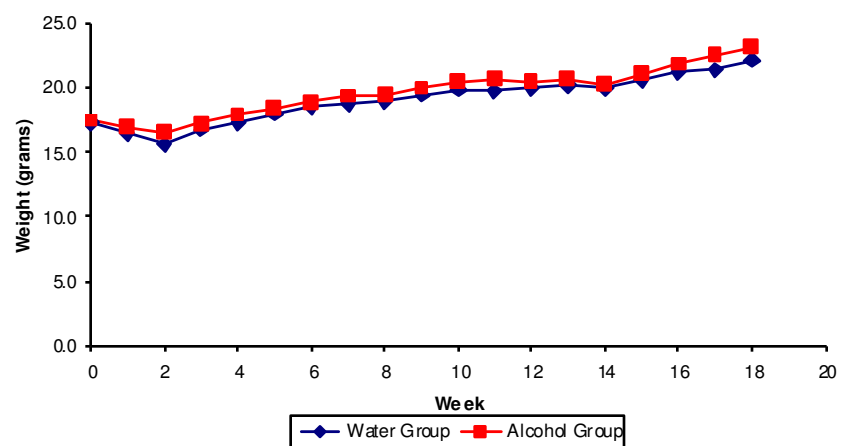


Figure 6a: Percentage body fat and lean body mass

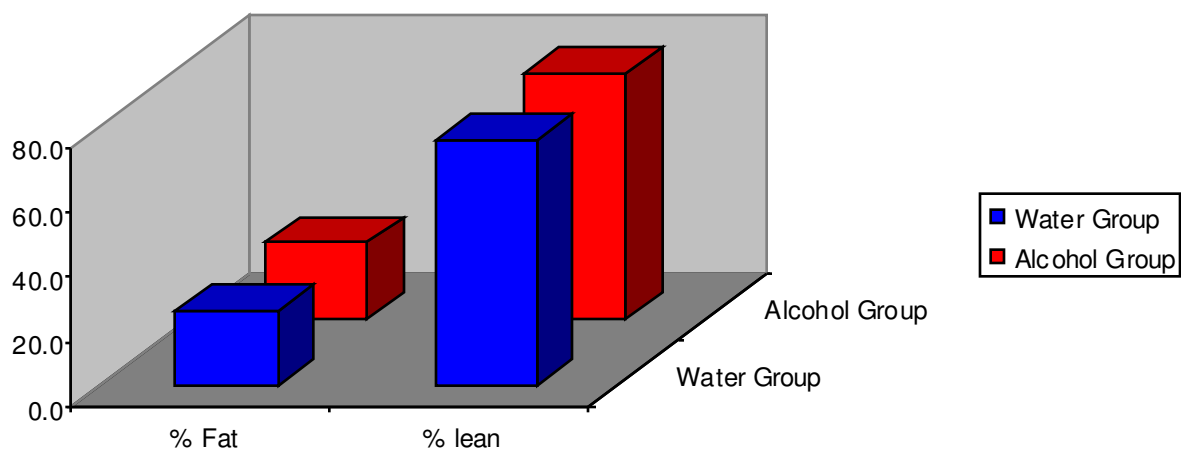


Figure 6b: Bone Mineral Density

